The Photochemical Formation of Formaldehyde in Green Plants.

By S. B. SCHRYVER, D.Sc., Ph.D., Lecturer on Physiological Chemistry in University College, London.

Since von Baeyer propounded his theory of the formation of formic aldehyde as an intermediate product in the synthesis of sugars from carbon dioxide and water by green plants, many attempts have been made to discover this substance, and to substantiate Baeyer's theory. The most successful of these are due to Polacci,[*] and to Usher and Priestley.[†] More recently R. J. Harvey Gibson, in conjunction with A. W. Titherley, has adduced further evidence tending to show that formaldehyde is actually formed in green plants, and the former has propounded a photo-electric theory to account for the photochemical action.[‡]

Nevertheless, the evidence hitherto brought forward has been generally considered, from an experimental point of view, somewhat inconclusive, and most of the above-quoted investigations have been subjected to numerous criticisms.

It is hardly necessary to examine these criticisms in detail here, nor to discuss the various theories that have been advanced to explain the mechanism of the reaction,[§] as it is thought that the experiments recorded in this paper will support an alternative hypothesis, by means of which some of the objections to the original conception of Baeyer may be removed.

Method.

During the course of some investigations on formaldehyde in foodstuffs[||] a method was devised by means of which both the free and combined aldehyde could be readily detected and quantitatively estimated, even when the amount present did not exceed one part in a million. The possession of this method afforded the opportunity for the re-investigation of the vexed question of the photochemical synthesis of the aldehyde by green plants.

[*] 'Arch. Ital. Biolog.,' vol. 35, p. 151, and vol. 37, p. 446.
[†] 'Roy. Soc. Proc.,' vol. 77, p. 369, and vol. 78, p. 318.
[‡] 'Annals of Botany,' 1908, vol. 32, p. 117.
[§] See Polacci, 'Accad. Lincei Roma,' June 6, 1908, and Ewart, 'Roy. Soc. Proc.,' 1908, vol. 80, p. 30.
[||] Report No. 9, Inspector of Foods Department of the Local Government Board.

The reaction employed for the purpose of estimation was, in its first form, originally described by Rimini, who showed that when formaldehyde solutions were treated with phenylhydrazine hydrochloride, and a drop of ferric chloride and concentrated sulphuric acid were subsequently added, a brilliant fuchsine-like colour developed. The reaction in this form was found to be uncertain; in the presence of too small a quantity of ferric chloride, the full colour due to the reaction was not developed, whereas in the presence of an excess of the reagent, the colour was rapidly destroyed. The use of concentrated sulphuric acid was also inconvenient when the reaction was employed quantitatively.

The reaction is due to the formation of a condensation product of formaldehyde and phenylhydrazine, which, on oxidation, yields a weak base; the latter, in the presence of excess strong acids, yields salts, which readily undergo hydrolytic dissociation on dilution. By substituting for ferric chloride an oxidising agent, which, when added in excess, does not destroy the colour, and by employing concentrated hydrochloric acid instead of sulphuric acid for the formation of the coloured salt, the Rimini reaction can be adapted to quantitative estimation of formaldehyde. In this form one part of formaldehyde in one million can be readily detected, whereas Rimini claimed that his original test was only sufficiently sensitive to detect one part in 50,000.

The test, with the above modifications, is carried out in the following way. To 10 c.c. of the formaldehyde-containing liquid are added 2 c.c. of a 1-per-cent. solution (freshly made up and filtered) of phenylhydrazine hydrochloride; 1 c.c. of a fresh 5-per-cent. solution of potassium ferricyanide is then added. On addition of 5 c.c. concentrated hydrochloric acid, a brilliant colour is developed in presence of formaldehyde; by comparing the depth of colour with that of standard solutions (of concentrations between 1 in 1,000,000 and 1 in 100,000), the quantity of formaldehyde present can be determined.

By the test in the above form, free formaldehyde can be detected and, when sufficiently dilute, quantitatively estimated. By a slight modification formaldehyde in a polymerised form, as in the so-called oxymethylene, or in the form of stable combination, as in hexamethylenetetramine (urotropine), can also be detected and estimated. If to a weak solution (10 c.c.) of hexamethylenetetramine be added phenylhydrazine hydro- and ferri-cyanide solutions, and then hydrochloric acid in the quantities given above, only a faint coloration (if any) will develop. If, however, the mixture be warmed for a short time, after addition of the phenylhydrazine hydrochloride solution, or be allowed to stand, and the ferri-

cyanide solution and acid be then added, the full colour due to the combined formaldehyde will develop ; the hexamethylenetetramine in the presence of water undergoes slight hydrolysis into ammonia and formaldehyde, and as the latter can be removed from the sphere of action in the presence of phenylhydrazine, the reaction in the presence of this reagent can proceed to completion, and the total combined formaldehyde can be thereby estimated. This reaction will be discussed in greater detail below, when considering the mechanism of the formaldehyde formation of chlorophyll.

In the presence of other pigments, the method of carrying out the reaction can be conveniently modified, especially when the quantities of formaldehyde present are only small. The reaction mixture, after addition of the reagents (phenylhydrazine and ferricyanide reagents and hydrochloric acid), is diluted with water in a small separating funnel, and ether is added. After shaking up, the hydrochloride of the chromatogenic base is dissociated, and the free base passes into the ethereal solution ; the latter is separated off from the underlying aqueous layer. On addition of concentrated hydrochloric acid to the ethereal solution, the base readily passes into the acid, in the form of the coloured hydrochloride. By using only a small quantity of acid, the sensitiveness of the test is largely increased, as the colour can be finally contained in only 1 or 2 c.c. of solution, instead of 18 c.c. as required in the original test. The test in this form has been applied in investigating the synthesis of formaldehyde by chlorophyll.

The test appears to be characteristic for formaldehyde. Commercial solutions of acetaldehyde yield a very faint pink colour, when in relatively strong solutions (1 to 2 in 1000), but this is due to contaminations with small quantity of formaldehyde, derived from methyl alcohol. Furfuraldehyde, which can certainly be obtained from plants, gives a characteristic reaction when present in sufficient concentration. On addition of phenyl-hydrazine and ferricyanide reagents to furfuraldehyde solution (1 in 1000) an apricot yellow colour is formed, which turns a dirty-green colour on addition of concentrated hydrochloric acid ; this, however, is only transient, and rapidly disappears. If, shortly after addition of the acid, and before the disappearance of the green colour, the reaction mixture be diluted and extracted with ether, and the ethereal solution be then extracted by acid, the latter takes up a green pigment. This reaction, however, is only obtained, if the dilution and extraction with ether be made very shortly after addition of the acid, and in this respect (as also in colour) it differs from the formaldehyde pigment, which is fairly stable. Furfuraldehyde, furthermore, gives no reaction with the reagents employed in the form-aldehyde test when the dilution reaches 1 in 100,000. The reaction, to be

described below, yielded by chlorophyll, can only be due, therefore, to formaldehyde.

Presence of Formaldehyde in Chlorophyll.

Grass was always employed as a source of chlorophyll in the following tests. It was first washed with warm water, and no formaldehyde was ever detected in the aqueous washings. It was then pressed by hand, to free it, as completely as possible, from the wash water, and extracted with methylated spirit. After standing for some time (generally over-night) the alcohol was filtered off on a Buchner funnel. Several times the alcoholic extract was tested directly for formaldehyde. It was evaporated to dryness, and the residue warmed with 10 c.c. of water and 2 c.c. of the phenylhydrazine reagent. The ferricyanide reagent and hydrochloric acid were then added. In no case did a colour develop. If, however, the alcohol were evaporated off *in vacuo*, and the residue taken up by ether, and the ether were then distilled off, a residue was obtained, which in certain cases gave a positive result when examined for formaldehyde. The reason for this behaviour was found to be due to the fact that the alcoholic extract contained substances, probably sugars, which interfered with the formaldehyde reaction, for after evaporating off the alcohol *in vacuo* at about 40° and extracting with ether, a residue insoluble in the latter solvent was obtained, which readily reduced Fehling's solution. For this reason, the chlorophyll must always be purified by solution in ether before carrying out the test.

In most of the samples of chlorophyll examined, formaldehyde was found. These were collected, however, during the summer season, after a long spell of daylight. A sample collected early on a foggy morning, after little sunshine, contained only a trace of formaldehyde.

In carrying out the test for formaldehyde with chlorophyll, the latter had been extracted with alcohol; this extract had been evaporated to dryness, and the residue thus obtained extracted with ether. The ethereal solution was itself evaporated to dryness. The formaldehyde reaction was then only obtained after allowing a film of the chlorophyll* obtained by evaporating the ethereal solution on a glass plate, either to stand for some time with a cold mixture of 10 c.c. water and 2 c.c. of a 1-per-cent. phenylhydrazine hydrochloride solution, or by warming the same mixture for a few minutes to 100° C. On addition of the ferricyanide solution and acid, the characteristic colour then developed.

The fact that formaldehyde could be detected in chlorophyll after repeated evaporation of the solutions, and that warming or standing with the phenyl-

---

* The crude product thus obtained is hereafter called chlorophyll, and is probably a mixture of several substances.

hydrazine reagent was necessary, indicate that the aldehyde is contained in the chlorophyll in the state of somewhat stable combination.

The Photochemical Formation of Formaldehyde by Chlorophyll.

In the earlier researches on this subject by Usher and Priestley, the formation of aldehyde was demonstrated by allowing chlorophyll films extended on gelatin plates, or on the surface of water, to remain exposed to light in the presence of carbon dioxide. It is conceivable that, in the experiments carried out in the first-named manner, some of the aldehyde may have been derived from the gelatin.* In the experiments described below, the formation of formaldehyde can be demonstrated when films of chlorophyll alone are employed.

It has been already stated that a sample of chlorophyll from grass collected on a foggy morning contained only very small traces of the aldehyde. Furthermore, formaldehyde gradually disappeared on keeping from ethereal solutions of chlorophyll, which were originally rich in the aldehyde. The experiments were carried out with such formaldehyde-free chlorophyll solutions.

Each test was carried out with 1 c.c. of an ethereal solution of chlorophyll, which was allowed to evaporate at room temperature on a strip of glass (about 140 by 20 mm.). Some films thus prepared were kept in the dark, others were exposed to moist carbon dioxide in sunlight, others to sunlight over lime or soda lime, and others, again, were exposed to moist carbon dioxide in the dark. For testing for formaldehyde two films were usually employed, and in each case either warmed for a few minutes or allowed to stand for some hours with a mixture of 10 c.c. water and 2 c.c. of the phenylhydrazine reagent, and the test was then completed in the usual manner. The results are tabulated below.

Conditions of experiment.	Result.
Kept in the dark either in presence or absence of moist CO_2	No formation of HCHO
Kept in sunlight over lime or soda lime	Formation of very minute quantities of HCHO
Kept in sunlight over moist CO_2..................	Formaldehyde reaction distinct

It will be seen that formaldehyde is formed when the chlorophyll film is exposed to sunlight either in presence or absence of carbon dioxide. In the latter case, the quantity of aldehyde formed is so minute that its presence could only be demonstrated with certainty after dilution of the reaction

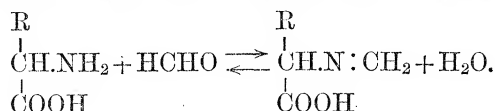* Compare Meisling, 'Revue Générale des Sciences,' May 15, 1909

mixture with water, extraction with ether, etc., in the method given above (see p. 228) for the modified formaldehyde test. In this case, carbon dioxide or formaldehyde was probably formed by the photochemical decomposition of other substances in the crude chlorophyll, for Neuberg has recently shown that a large number of organic substances readily undergo decomposition on exposure to light in the presence of optical sensitisers, such as uranium salts.* When carbon dioxide was present, the formaldehyde reaction was visible when the ordinary test was applied, without having to dilute with water and extract the chromatogenic base with ether. In no case, however, was the formation of formaldehyde demonstrable when the films were kept in the dark. These experiments were carried out several times with different chlorophyll preparations, and yielded always the same results. Only very small quantities of chlorophyll need be employed in each experiment. In the quantities employed for each of the experiments described above, no formaldehyde could be detected in the original film by any of the given methods of testing.

Mechanism of the Reaction.

It has been generally objected to the conception of the formation of formaldehyde as an intermediate product of sugar synthesis in plants, that this substance is highly toxic. This objection has, however, lost some of its weight as a result of the experiments of Tréboux,† who states that certain plants, when placed in very dilute solutions of formaldehyde, can synthesise therefrom starch.

The fact that formaldehyde after synthesis by chlorophyll exists in the state of fairly stable combination with some product or group, explains how the quantity of the aldehyde present can be automatically regulated. The action of formaldehyde on various organic substances, especially those containing the amino-group, has been studied by Schiff.‡

He has shown that in many cases the reaction between an amino-group and the aldehyde is a reversible one, as in the case of the amino-acids—

$$\begin{matrix} R \\ | \\ CH.NH_2 \\ | \\ COOH \end{matrix} + HCHO \rightleftarrows \begin{matrix} R \\ | \\ CH.N:CH_2 \\ | \\ COOH \end{matrix} + H_2O.$$

In some cases this reaction proceeds to completion only in the presence of a large excess of the aldehyde.§ In other cases, as in that of

* 'Biochem. Zeitsch.,' 1908, vol. 13, p. 305.

† 'Flora,' 1903, p. 73.

‡ 'Liebig's Annalen,' 1899, vol. 310, p. 25 ; 1901, vol. 319, pp. 58 and 287 ; and 1902, vol. 325, p. 348.

§ Compare Sörensen, 'Biochem. Zeitsch.,' 1908, vol. 7, p. 45.

hexamethylenetetramine, the hydrolysis of the methyleneamino-derivative, which is a somewhat stable compound, is only very small (the reaction represented by the arrow pointing to the left in the above equation is only very incomplete). In such cases, the formaldehyde reaction can only proceed to completion after warming or allowing the mixture to stand for some time in the presence of the phenylhydrazine reagent. The hydrolysis proceeds to completion, that is to say, when the formaldehyde that is set free is removed from the sphere of action. A similar stable compound appears to be formed with the chlorophyll* and formaldehyde. The reaction may be schematically represented by the following equation :—

$$\text{Chlor.} \diagup^{H}_{\diagdown H} + HCHO \rightleftharpoons \text{Chlor.} \diamondsuit CH_2 + H_2O.$$
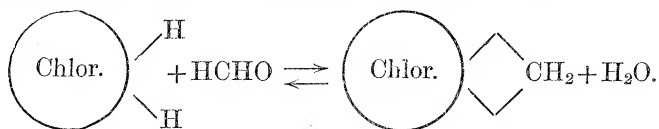
As the condensation product is somewhat stable, equilibrium will be maintained when only a very small amount of free aldehyde is present. As this is removed by synthesis into sugars, etc., more of the chlorophyll-aldehyde condensation product will decompose. It can furthermore be reconstituted in the presence of carbon dioxide and sunlight. The fact, therefore, that such a stable compound is formed, which undergoes only limited hydrolysis, explains the mechanism by means of which the quantity of formaldehyde in the plant available for sugar synthesis can be regulated. In the presence of sunlight and carbon dioxide, there is probably a continuous synthesis of formaldehyde, and a continuous condensation of the latter to sugars, without at any time an accumulation of the aldehyde in such quantity as to be toxic to the plant. It is possible that the synthetic formation of sugar is due, as Usher and Priestley have suggested, to the living protoplasm, as this hypothesis explains certain phenomena to which they have called attention in their paper.

Summary.

1. A reaction is described by means of which minute quantities of formaldehyde, both combined and free, can be detected and estimated.

2. Formaldehyde can be detected in chlorophyll, and exists therein in a state of combination.

3. By means of the reaction described, the photochemical synthesis of formaldehyde by chlorophyll can be demonstrated.

4. From the fact that formaldehyde exists in a state of combination in chlorophyll, it is possible to explain how the supply of aldehyde necessary for sugar synthesis in the plant is regulated.

* See note, p. 229.